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Isolation, Screening and Biochemical Characterization of Used Engine Oil Degrading *Bacillus* Species and *Pseudomonas* Species

Abdullahi Aliyu Jibo[1], Kabiru Ibrahim Karamba[1*]

[1]*Department of Microbiology, Faculty of Science, Bauchi State University Gadau P.M.B 065, Bauchi, Nigeria*.

**Correspondence: kabirukaramba@basug.edu.ng +2348065927314*

Abstract

Extensive demand of natural resources has resulted in several large-scale unintentional hydrocarbon oil spills and environmental catastrophes due to the rising demand for fossil fuel energy. These hydrocarbon pollutants have effect on environment and human health. Spectrophotometric and Biochemical Methods are used for the research. Soil sample were collected from two different contaminated areas and used engine oil, the bacteria were isolated and tested for evaluation of bacterial growth and biodegradation by enrichment technique using Bushnell Hass broth with used engine oil as sole carbon source. The results show that *Pseudomonas* specie were capable of degrading used engine oil which remove 80% of the oil and *Bacillus* specie which remove 65% of the used engine oil. This illustrates that hydrocarbon biodegrading bacteria can be used for remediation of hydrocarbon contaminated soil.

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1.0 Introduction

Hydrocarbon spills, such as those caused by used engine oil, can have negative effect on people, animals, plants, and ecosystems that can only be remedied by using traditional disposal techniques like incineration, thermal conversion, land filling, and paralysis techniques *(Adeleye et al*., 2018). Contrarily, using bio-stimulation or bio-augmentation techniques, bioremediation can completely degrade pollutants (Ajiboye *et al*., 2020). The environmental hazards posed by petroleum hydrocarbons are severe (Kawo and Faggo, 2017). Major hazards from oil spills are mostly seen in industry and undeveloped nations (Musa, 2019) that pose a serious risk to the nearby ecosystems (Head *et al*., 2006). These dangers impair soil fertility, disrupt soil-borne bacteria, and harm plants (Lovett *et al*., 2009). Conversely, prolonged exposure to high oil concentrations may increase the risk of cancer, liver or renal illness, and even bone marrow degeneration *(Valentin et al*., 2006). There are potentially dangerous and mutagenic chemicals present; consequently, the Environmental Protection Agency (EPA) categorized crude oil as serious pollutants (Liu *et al*., 2016).

In the course of processing, moving, or storing oil, contamination may happen accidentally or intentionally (Eniola *et al*., 2014). The primary and dependable method for removing thousands of environmental pollution, including crude oil, from the environment is biodegradation by intrinsic microbial communities (Shahriari Moghadam *et al*., 2014).

Numerous bacteria called petrophiles have the ability to use hydrocarbons and naturally decompose heavy hydrocarbons (Obayori *et al*., 2009).

2. 0 Materials and Methods

2.1 Collection of Soil Sample

The blackish soil samples were sampled from two separate oil-contaminated sites: the first from Tunde Automobile Workshop within Babban Gareji Azare, and the second from Baffa Automobile Workshop area adjacent Bilal Academic Azare in Katagun Local Government Area of Bauchi State. The samples were collected at each location by digging up the soil with a spade for about 10 cm, transferring them straight into a sterile polyethene bag stored in ice cold box and transported to the laboratory, where they were maintained at 4°C until utilized for the isolation of used motor oil degrading bacteria.

2.2 Used Engine Oil (UEO)

Used engine oil was taken in glass bottles with screw caps from Alhaji Ubale Engine Oil inside Babban Gareji Azare. The oil was transported to the lab and kept in the refrigerator until usage. It was a dark, almost black brown tint in color.

2.3 Medium Composition

The isolation of bacteria that break down spent motor oil was done using Bushnell Haas (BH) broth medium. With the exception of a carbon source, it has the following medium composition and nutrients: $MgSO_4.7H_2O$ (0.2 g/L), K_2HPO_4 (1.0 g/L), KH_2PO_4 (1.0 g/L), $FeCl_3$ (0.05 g/L), NH_4NO_3 (1.0 g/L), and $CaCl_2$ (0.02 g/L) with a pH of 7.2.

2.4 Isolation of Bacteria from Used Engine Oil Contaminated Soil

Serial dilutions were made for the samples from 10^1 to 10^6 dilutions, and the diluted samples were inoculated using a sterile wire loop into Nutrient agar plate and it was incubated at 37ºC for 24 hours. Bacterial colonies were isolated and sub-cultured onto separate nutrient agar plates. The pure cultures were incubated at 37°C for 24 hours. Pure isolate obtained were further identified using the following test; Gram stain, catalase, oxidase, urease, indole and coagulase test.

2.5 Preparation of Standard Calibration curve

Bushnell Haas Broth ingredients were dissolved in 0.2, 0.4, 0.6, 0.8, and 1.0 milliliters of used motor oil, respectively, to make 100 milliliters of the broth. The media were autoclaved for 20 minutes, at 15 psi. A spectrophotometer was used to detect absorbance at 272 nm, and the results were recorded (Karamba and Ahmad, 2019).

2.6 Screening of Bacterial Isolates for the Degradation of Used Engine Oil

Ninety Six Milliliter (96 ml) of Bushnell Haas Broth were dissolved in 2 ml of used motor oil and media were autoclaved at 15 psi for 20 minutes. A 2 ml dose of the recognized grown culture that was aged for 24 hours was put into cooled medium. The flasks were incubated for 192 hours at 28 °C with an orbital shaker spinning at 160 rpm (Maarof *et al*., 2018).

2.7 Analytical Methods

2.7.1 Evaluation of Bacterial Growth

The rate of bacterial growth was assessed as previously described by Karamba *et al*., (2018) UV-V spectrophotometer were used to measure the optical density ($O.D_{600}$).

2.7.2 Determination of Residual Engine Oil

Every 24 hours, samples were collected for analysis, and the results were assessed using a spectrophotometer according to the Ibrahim *et al*. (2016), A UV-V spectrophotometer was used to measure the absorbance at 272 nm after each sample had been centrifuged at 10,000 x g for 15 minutes

3.0 Results and Discussion

3.1 Isolation of Bacteria from Used Engine Oil Contaminated Soil

Four (4) bacterial isolates (Table 1) with an apparent clear zone were selected from each contaminated soil sample. They are found to be effective in the remediation of Used Engine Oil.

3.2 Standard Calibration Curve

In order to ascertain the unknown concentration of used engine oil, the absorbance of engine oil concentration ranges from 0.2 ml to 1.0 ml was measured in a spectrophotometer at 272_{nm} (Fig1).

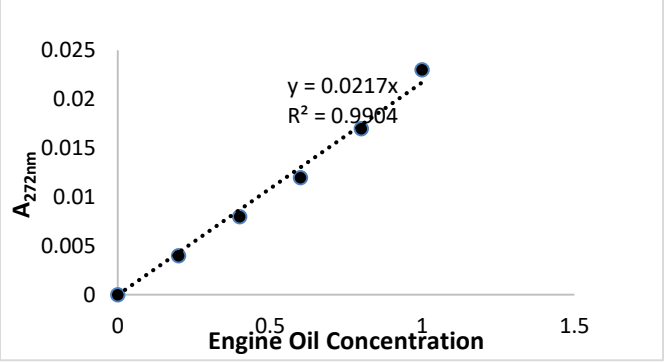

Fig: 1. Standard calibration curve for engine oil concentration

S/N	Name of Oil Contaminated Soil Site	Bacterial Isolates			
Sample 1	Tunde Automobile Workshop (TAW), Azare	A	B	C	D
Sample 2	Baffa Automobile Workshop (BAW), Azare	E	F	G	H

3.3 Bacterial Growth

Following 192 hours of incubation, isolates A, B, C, D, E, F, G, and H showed different levels of bacterial growth in relation to the biodegradation of engine oil: isolate A: 0.772, isolate B: 0.567, isolate C: 0.990, isolate D: 1.040, isolate E: 1.030, isolate F: 0.967, isolate G: 1.640, and isolate H: 0.640. These findings show that the maximum bacterial growth at $O.D_{600nm}$ is found in isolates G from BAW bacteria and D from TAW bacteria, whereas the weakest bacterial growth at $O.D_{600nm}$ is found in isolates B from TAW bacteria and H from BAW bacteria (Fig. 2).

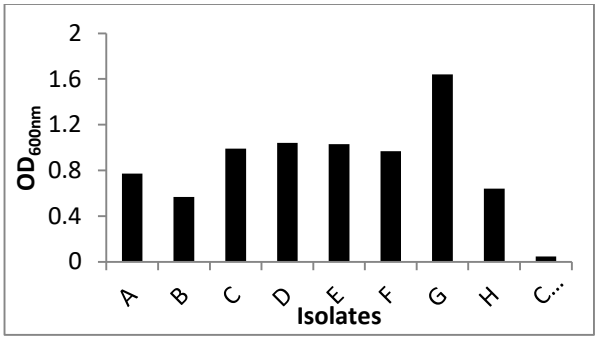

Fig: 2. Bacterial Growth in Relation to Engine Oil Biodegradation after 192 hrs Incubation

3.4 Biodegradation of Used Engine Oil

Fig. 3 illustrates the microbial degradation of used engine oil by TAW bacteria at 272 nanometers using a UV-V spectrophotometer. The remaining concentrations of used engine oil after 192 hours of incubation are isolates A= 0.910, B= 1.130, C= 0.822, and D= 0.514, respectively. Additionally, isolate D exhibits the highest biodegradation with 65% degradation of used engine oil and isolate B exhibits the lowest degradation with 23%.

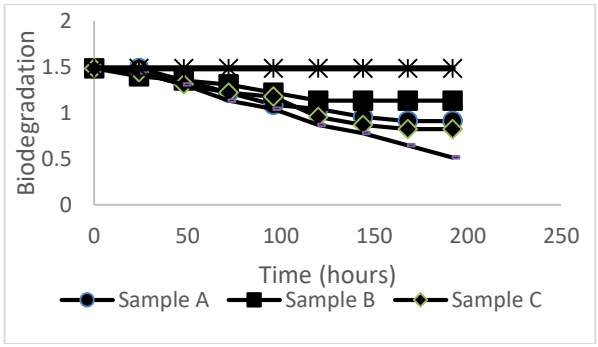

Fig: 3. Biodegradation of Engine Oil by TAW Bacteria

3.5 Biodegradation of Used Engine Oil by BAW

Biodegradation of engine oil after incubation for 194 hours by BAW bacteria was measured at 272_{nm} using a UV-V spectrophotometer to determine the remaining concentration of engine oil isolate E= 0.690, isolate F= 0.822, isolate G= 0.294, and isolate H= 1.042. Isolate G also had the highest degradation with 80% degradation of used engine oil and isolate H had the lowest degradation with 29% degradation of used engine oil (Fig. 4).

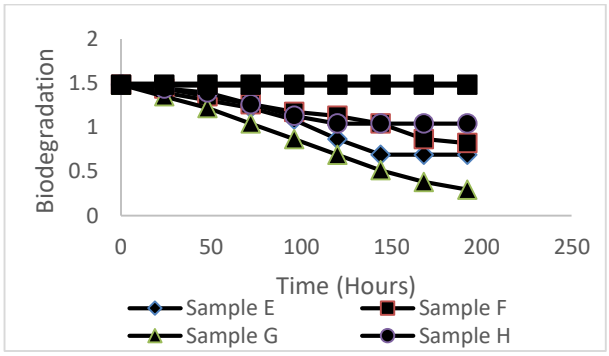

Fig: 4. Biodegradation of Engine oil by BAW Bacteria

3.6 Biochemical Identification of Selected Bacterial Isolates

According to Figures 2, 3, and 4, of the eight bacterial isolates, Isolate D and Isolate G most effectively

exploited used engine oil as the only source of carbon and energy and demonstrated superior growth and degradation thus were used to proceed with further research. (Karamba *et al.*, 2015: Ibrahim, 2016).

According to Figures 2, 3 and 4 respectively, it is evident that each bacterial isolate will experience a different level of bacterial growth depending on the degree of degradation. In order to identify specific bacterial isolates, gram staining and biochemical assay were used, as shown in the **Table 2** below. The results illustrated that the suspected bacteria responsible for the biodegradation are *Pseudomonas* species, *Bacillus* species where the former have the capacity to remove 80% of the engine oil and the latter 65%. These goes in tandem with the growth of bacteria in which *Pseudomonas* Specie have highest proliferation of 1.640 in optical density value followed by *Bacillus* Specie with 0.514.

Table: 2. Gram Reaction and Biochemical Characterization of Used Engine Oil Degrading Bacterial Isolates

S/N	Tests	Isolate D	Isolate G
1	Gram Reaction	+	-
2	Cell Shape	Rod	Rod
3	Catalase	+	+
4	Indole Production	-	-
5	Urease	-	-
6	Oxidase	-	+
7	Coagulase	-	-
8	Presumptive Organism	*Bacillus* specie	*Pseudomonas* specie

Key: (+) = Positive and (-) = Negative

4.0 Conclusion

In this work the two (2) bacterial isolates biodegraded used engine oil were isolated from automobile polluted soil. The suspected species identified to biodegrade used engine oil effectively are *Pseudomonas* specie, *Bacillus* specie. These results show that these native bacteria has the ability to utilise hydrocarbons as carbon source in oil-polluted locations, which may be improved by optimization (Karamba *et al*., 2016; Karamba and Abdullahi, 2022) to enhance bioremediation in areas that has been exposed to oil for a long time.

Declarations

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Ethics approval and consent to participate

Not Applicable.

Consent for publication

All authors have read and approved the final draft of the manuscript.

Availability of data and material

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

AAJ and KIK conceptualized the design of the study. AAJ conducted the research under the supervision of KIK and both authors prepared the first draft of the manuscript. All authors read and approved the final manuscript.

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